

Realtime qPCR for quantification

RT qPCR for amplification of the specific tRNA species transcribed into cDNA by means of a specific primer:

Performing the analysis in specific white qPCR plates

1. Set up reaction as follows:

Table 1: Representation of the composition for RT qPCR

Component	reaction
specific cDNA of corresponding tRNAs	5 µL
Primer forward (10pmol/µl)	0,4 µL
H ₂ O	4,2 µL
specific tRNA Primer (10pmol/µl)	0,4 µl
GreenMasterMix 2x (Genaxxon)	10 µl

For the negative control 5 µl of H₂O are used instead of above-mentioned primers and H₂O.

2. Executing the following program in the qPCR Tower (qTOWER³, Analytik Jena)

Table 2: Representation of temperatures, times and cycle numbers for RT qPCR

Period of time	temperature	number of cycles	
10 min	95 °C	} 45x	
15 s	95 °C		
15 s	55 °C		
15 s	72 °C		